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A comparative study of fluoride ingestion levels, serum thyroid hormone & TSH level derangements, dental fluorosis status among school children from endemic and non-endemic fluorosis areas

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Abstract

The study was undertaken to determine serum/urinary fluoride status and comparison of free T₄, free T₃ and thyroid stimulating hormone levels of 8 to 15 years old children with and without dental fluorosis living in an endemic and non-endemic fluorosis area. A sample group of 60 male and female school children, with or without dental fluorosis, consuming fluoride-contaminated water in endemic fluoride area of Udaipur district, Rajasthan were selected through a school dental fluorosis survey. The sample of 10 children of same age and socio-economic status residing in non endemic areas who did not have dental fluorosis form controls. Fluoride determination in drinking water, urine and blood was done with Ion 85 Ion Analyzer Radiometer with Hall et al. method. The thyroid gland functional test was done by Immonu Chemiluminiscence Micropartical Assay with Bayer Centaur Autoanalyzer. The significantly altered FT₃, FT₄ and TSH hormones level in both group1A and 1B school children were noted. The serum and urine fluoride levels were found to be increased in both the groups. A significant relationship of water fluoride to urine and serum fluoride concentration was seen. The serum fluoride concentration also had significant relationship with thyroid hormone (FT₃/FT₄) and TSH concentrations. The testing of drinking water and body fluids for fluoride content, along with FT₃, FT₄, and TSH in children with dental fluorosis is desirable for recognizing underlying thyroid derangements and its impact on fluorosis.

Keywords: Fluorosis; Water; Thyroid; Udaipur

Introduction

Iodine Deficiency Disorders (IDD) and fluorosis are the two most prevalent endemic diseases which coexist in certain regions in India (Hetzel et al. 1990). As early as 1928, Stocks (1928) observed that children consuming well water in the village of Somerset, England exhibited both goiter and mottled enamel (dental fluorosis). Some years later, Wilson (1941) found dental fluorosis (DF) associated with goiter and cretinism among children living in areas of Punjab where fluoride was recognized geologically to be significantly high. Besides dental fluorosis and cretinism,

children in endemic fluorosis areas of India often have low IQ, deaf mutism, knock-knee and bow-legs (Susheela 2003). The DF is a developmental disorder which is due to aberrant thyroid hormone metabolism. These are serious public health problems of great concern. Since fluoride is known to interfere with thyroid gland function and to cause degenerative changes in the central nervous system, impairment of brain function and abnormal development in children (Ha et al. 1989), further investigation is clearly needed, even where iodine intake is not deficient.

As it is already known that fluoride is more electronegative than iodine, it easily displaces iodine within the body, thereby affects the functioning of thyroid gland. Fluoride has been known to have shown gross as well as biochemical changes within the body of an individual which included deranged thyroid hormonal level within the body. The production of thyroid hormones is regulated by a negative

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Table 1 Levels of fluoride naturally ingested from drinking water and body fluids in different sample groups

Parameters	Group 1A	Group 1B	Group 2	Total
Water fluoride (WF)	1.6–5.1 ppm	1.6–5.5 ppm	0.98–1 ppm	0.98–5.5 ppm
Urine fluoride (UF)	0.24–8.9 ppm	0.4–7.79 ppm	0.19–1.01 ppm	0.24–8.9 ppm
Serum fluoride (SF)	0.02–0.77 ppm	0.03–0.75 ppm	0.02–0.09 ppm	0.02–0.77 ppm

feedback mechanism, i.e., when the pituitary gland senses a drop in FT_3 levels in circulation, it releases more TSH to stimulate the thyroid gland which in turn accelerates the production of the thyroid hormone T_4 , now considered a “pro-hormone”. The major source of circulating T_3 is from peripheral deiodination of T_4 and not from thyroid secretion. The enzymes which catalyze deiodination are called iodothyronine deiodinases and fluoride is known to interfere with the activity of the deiodinases.

The effect of the fluoride on tooth development is dose dependent. Their effects on various stages of tooth development, on dental tissues and on enamel in particular have been related to the interaction between fluoride ions and calcium hydroxyapatite besides the regulatory effects of thyroid secretions. To gain a better understanding of the problem, the present study was undertaken to determine the fluoride status and compare it with the free T_4 , free T_3 , and thyroid stimulating hormone (TSH) levels of children with and without dental fluorosis living in an endemic fluorosis area.

Materials and method

The study was conducted to evaluate and correlate the effect of chronic excess fluoride intake on thyroid function among school children (8–15 years) from endemic and non-endemic fluorosis areas. The villages with high fluoride levels in the drinking water in the Udaipur district of Rajasthan, India were included as endemic areas (Group 1). The villages which were selected were Slumber, Sarada, Kalutada, Devgaun and Kejad. Group 1 included 60 male and female school children, which were equally divided into two subgroups: Group 1A (children with dental fluorosis) and Group 1B (children without dental fluorosis).

Group 2 included 10 children from Sardarpura colony of Udaipur city, a non endemic area, which was taken as a control for the study samples.

It has been reassured that the study was conducted in conformity with principles in Helsinki Declaration and others specified in the respective ethics committees of Rajasthan University. In group 1A ($n = 30$), 15 children

were selected from areas having water fluoride level up to 2.6 ppm while the remaining 15 children were from areas having water fluoride level up to 5.1 ppm. In group 1B ($n = 30$), 15 children were selected from areas having water fluoride level up to 2.6 ppm while the remaining 15 children were from areas having water fluoride level up to 5.1 ppm. The group 2 children ($n = 10$) were of the same age range and socio economic status, residing in the non-endemic area, without exhibiting dental fluorosis and serving as the control group. Their drinking water was deemed to be “safe” (<1.0 mg F-/L) and like the sample group, these control children were also investigated for thyroid gland function.

The school children’s dental history with dentition status and Dean’s index was recorded. The six grades are known as Dean’s classification scale for dental fluorosis. They are: none (normal enamel) (the score marked for 0), suspected or questionable (0.5), very mild (1), mild (2), moderate (3), and severe (4). Statistical analysis of the prevalence of dental fluorosis was made according to the rates of DF%. The morning blood sample was collected of these children by venipuncture into the tubes on the day before and serum was separated via centrifuge, for thyroid gland function tests and fluorine estimation in serum. Serum fluoride concentration is recognized as a good indicator of fluoride exposure. The samples were stored in refrigerator at 4°C and then analyzed for the fluoride levels. The drinking water fluoride estimation was done to adjudge the endemic and non-endemic areas. The Urinary fluoride is regarded as an indicator of grade of exposure to fluorine compounds absorbed.

Method

The samples of drinking water, urine and blood were collected in plastic bottles, blood samples were left to clot at room temperature and the serum was separated by centrifugation (Negoiita et al. 2001). Fluoride ion level in body fluid was estimated by manual titration method and automatic fluoride Ion 85 Ion Analyzer and radiometer with Hall et al. in (1972) and ASTM method. Fluoride

Table 2 Levels of thyroid hormones in all sample groups

Parameters	Group 1A	Group 1B	Group 2	Total
Free T_3 (FT_3)	1.1–4.39 pg/ml	1.2–4.57 pg/ml	1.90–4.13 pg/ml	1.1–4.57 pg/ml
Free T_4 (FT_4)	0.94–1.98 ng/dL	0.8–1.7 ng/dL	0.87–1.67 ng/dL	0.8–1.98 ng/dL
TSH	1.41–8.46 $\mu\text{IU}/\text{m}$	1.92–10.99 $\mu\text{IU}/\text{m}$	0.96–3.54 $\mu\text{IU}/\text{m}$	0.96–10.99 $\mu\text{IU}/\text{m}$

Table 3 Derangement in Thyroid hormone (FT₃, FT₄, TSH) levels and serum fluoride levels in children of different groups

Group	No. of cases with Derangement in Thyroid hormone (FT ₃ , FT ₄ , TSH) level	No. of children with abnormal serum fluoride level	No. of children with delayed eruption
Group1A (n = 30)	23 (77%)	29 (97%)	17 (57%)
Group1B (n = 30)	20 (67%)	30 (100%)	15 (50%)
Group 2 (n = 10)	1 (10%)	5 (50%)	0 (0%)

determination in the drinking water, urine and blood serum was carried out potentiometrically with an Ion 85 Ion Analyzer and a fluoride ion specific electrode and radiometer with ASTDM method. The serum samples of children were investigated to assess FT₄, FT₃, and TSH hormone levels using Immuno Chemiluminiscence Microparticle Assay (ICMA) with the Bayer Centaur Autoanalyzer.

Results

All the data was statistically analyzed using SPSS 13 software. The level of fluoride naturally ingested from drinking water and body fluids in all sample groups which is varied over a wide concentration range of fluoride content in drinking water from 0.98 to 5.5 mg F⁻/L (ppm), in blood serum 0.02–0.77 mg F⁻/L (ppm) and in urine from 0.24–8.9 mg F⁻/L (ppm). (Table 1). Table 2 showed the thyroid (FT₃ & FT₄) and TSH hormone level in body fluids for all 60 children of group 1A and 1B and 10 children of Group 2. FT₃ levels were recorded highest in Group 2 with minor difference in other groups; concentration of FT₄ levels was maximum in Group 1A, whereas TSH levels were significantly higher in Group 1B.

The thyroid hormone (FT₃, FT₄, TSH) levels revealed that the derangements are present in 72% of cases in group 1, whereas only 10% of children were involved in group 2. 50% children in group 2 had very slightly

elevated serum fluoride concentration, whereas in group 1, 98% of children were involved. The children of endemic areas revealed derangement of serum thyroid hormone and TSH levels, along with increased fluoride concentration in the body fluids. This affects the tooth development in the form of delayed eruption of teeth in the children of endemic area but not in the non-endemic area (Table 3).

Table 4 indicates the correlation of various parameters with different groups. A significant correlation was found with values of TSH in different groups; whereas FT₃, FT₄ levels were insignificantly correlated. Level of water, serum and urine fluoride was showing highly significant correlation with both group 1 & 2.

Dean's Fluorosis index was used to grade level of fluorosis in children of Group 1A. The results showed mild fluorosis in 13% children, moderate fluorosis in 67% children and severe fluorosis in 20% children, suggesting that the influence in endemic fluoride area on tooth formation is highly significant & the least effect is also so much that it causes moderate dental fluorosis as a consequence of fluoride toxicity (Table 5).

Table 6 showed the correlation of fluoride content in body fluids and their effect on thyroid hormones within group 1 (fluoride endemic area). Water fluoride level has very highly significant correlation with serum fluoride level, urine fluoride level and FT₃ hormone level, whereas a

Table 4 Correlation of various parameters with Group 1 and Group 2

Parameters	Group	n	Mean	Std. Deviation (S.D)	t-test	df	p-value*
FT ₃	1	60	3.0758	1.10440	1.589	68	0.117
	2	10	2.5000	0.71146			
FT ₄	1	60	1.1982	0.21692	0.259	68	0.796
	2	10	1.1790	0.21845			
TSH	1	60	3.7062	1.93841	1.940	68	0.057*
	2	10	2.4960	0.75167			
WF	1	60	2.685	1.1970	4.453	68	0.000*
	2	10	0.989	0.0099			
UF	1	60	2.6518	2.04664	3.058	68	0.003*
	2	10	0.6582	0.25726			
SF	1	60	0.1863	0.15833	2.878	68	0.005*
	2	10	0.0410	0.02807			

*Correlation is significant at the 0.05 level (2-tailed t-test).

Table 5 Children of group1A with Dean's grades of fluorosis

Dean's fluorosis index grades	Percentage of children in Group 1A
0	0%
1	0%
2	13%
3	67%
4	20%

significant correlation was found with FT₄ level. Serum fluoride level has a highly significant correlation with FT₃ and TSH. FT₃ has highly significant correlation with TSH and significant correlation with FT₄ hormone level.

Discussion

Fluoride and iodine are both halogens. The fluoride, the negative ion of the element fluorine easily displaces iodine in the body because it is much lighter and therefore more reactive. In fact the activity of any one of the halogens is inversely proportion to its atomic weight. In other words, one halogen can displace another one of a higher atomic weight but cannot displace one of lower weight thereby, results fluoride- thyroid-iodine antagonism which in turn lead to interference with iodine uptake. The fluoride is a universal G-protein activator/inhibitor. The stimulation of certain G-proteins occurs due to the toxic effects of fluoride, which has the effects of switching off the uptake into the cell of the active thyroid hormone. The thyroid control mechanism is compromised. The TSH output from pituitary gland is inhibited by fluoride, thus reducing thyroid output from thyroid glands. Fluoride competes for the receptor sites on the thyroid gland which respond to TSH; so that this hormone reaches the thyroid gland and so fewer

hormone is manufactured (Wilson and DeEds 1940; Susheela et al. 2005).

The enzymes which catalyze deiodination are called iodothyronine deiodinases, of which three have been identified as D₁, D₂, and D₃ (Visser and Peeters 2012). D₁ activity is responsible for conversion of T₄ to T₃ in peripheral tissues, particularly in the liver and is reflected in plasma T₃ levels. D₁ activity has both outer ring deiodination as well as inner ring deiodination activity. Among the three deiodinases, D₁ is expressed in thyroid gland besides the liver and kidney. D₂ is found in brain, pituitary gland, and skeletal muscle and D₃ is highly expressed in brain, placenta and fetal tissues. The present study has focused on thyroid hormones rather than the deiodinases, fluoride is known to interfere with the activity of the deiodinases.

In the present study, we found statistically significant relationship between water fluoride-urine fluoride and water fluoride-serum fluoride. Similar relationships were also observed by Rathee et al. in (2004). In another study conducted by Xiang et al. in (2009), the significant positive relation between serum fluoride and drinking-water fluoride was found. In our study, we found significant relationship between serum fluoride and TSH, serum fluoride and FT₃/FT₄, TSH and FT₃/FT₄. Similar significant relationship was also found by Susheela et al. (2005) and Wu et al. (2008). We observed that high fluoride exposure can cause functional abnormalities of thyroid and different degrees of dental fluorosis could be observed with significant deviation in the serum thyroid hormone levels, as cited by Xiang et al. (2009).

When serum FT₃, FT₄ and TSH of group 1 and group 2 of our study were compared we found significant difference between the two groups in serum TSH; FT₃-FT₄; TSH-FT₃; TSH-FT₄. The serum TSH, TSH-FT₃ and TSH-FT₄ were significantly higher unlike the results

Table 6 Correlation analysis between fluoride content in body fluids and their effect FT₃, FT₄, TSH within Group 1

Parameters	Spearman rho analysis	FT ₃	FT ₄	TSH	WF	UF	SF
FT ₃	'r'	1	-0.169	-0.252	-0.711	-0.388	-0.400
	p-value	-	0.196	0.052*	0.000**	0.002**	0.002**
FT ₄	'r'	-0.169	1	-0.079	0.196	0.119	0.119
	p-value	0.196	-	0.547	0.134	0.365	0.366
TSH	'r'	-0.252	-0.079	1	0.151	0.079	0.552
	p-value	0.052*	0.547	-	0.250	0.550	0.000**
WF	'r'	-0.711	0.196	0.151	1	0.690	0.529
	p-value	0.000**	0.134	0.250	-	0.000**	0.000**
UF	'r'	-0.388	0.119	0.079	0.690	1	0.525
	p-value	0.002**	0.365	0.550	0.000**	-	0.000**
SF	'r'	-0.400	0.119	0.552	0.529	0.525	1
	p-value	0.002**	0.366	0.000**	0.000**	0.000**	-

*Correlation is significant at 0.05 levels (2 tailed), **Correlation is highly significant below 0.01 levels correlation coefficient(r).

of study in rats in which contrary value of FT_3/FT_4 were found (Brtko et al. 1993).

In view of serum and urinary fluoride levels in the Group 2 in the present study, wherein the likelihood of fluoride ingestion from food and other sources is apparent. Hence, it is evident that analysis of fluoride in body fluids besides drinking water is highly relevant and necessary for understanding potential health implications.

The chronic over exposure of fluoride in drinking water causes growth disturbances particularly evident in adolescence and they result in thyroid dysfunction as studied by various authors, for the same reasons we also see dentition disturbances like dental fluorosis (Smith and Tafforeau 2008; Villa et al. 2010). Hence, it is evident that the analysis of fluoride in body fluids besides drinking water is highly relevant and necessary for understanding potential health implications (Martins et al. 2011). The results of the present investigation indicate that improvement in the health of the children would likely be achieved if management strategies incorporate emerging knowledge to address fluoride toxicity in the individuals, even if residing in non endemic fluoride areas.

Conclusion

The results of this study question the validity of the fluoridation of drinking water, milk, fruit juices, and salt by public health authorities and also the step taken to prevent ill effects of excess fluorine and iodine deficiencies in endemic fluorosis areas. The children with dental fluorosis living in endemic fluorosis areas may not have a frank thyroid disease due to excessive fluorine consumption but they do show thyroid disease leading to many health effect hence they require special care and attention.

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

The manuscript has been read and approved by all the authors, the requirements for authorship as stated earlier in this document have been met, and each author believes that the manuscript represents honest work.

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